Evaluating the Use of Carbon Dioxide as an Alternative Predator Removal Technique to Decrease Tracy Fish Collection Facility Predator Numbers and Improve Facility Operations

Investigators

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Summary

The Tracy Fish Collection Facility (TFCF) was developed in 1956 by the U.S. Bureau of Reclamation (Reclamation) as a means of salvaging fish greater than 20 mm FL and returning them to various points downstream on the Sacramento-San Joaquin River Delta (SSJD) beyond the influence of C.W. "Bill" Jones Pumping Plant (JPP). To improve the overall salvage process and efficiency of the TFCF we need to minimize fish losses throughout the facility. Many factors, including predation, contribute to the total fish loss at the TFCF. Predators accumulate throughout the facility, including in front of the trashrack, the primary channel, the bypass tubes, the secondary channel, and the holding tanks (HT; Liston *et al.* 1994). Length vs. weight relationships developed with fish collected from the TFCF suggest that, although a 57-mm spaced bar trashrack heads the facility, striped bass (*Morone saxatilis*) up to 508 mm FL can still pass through and set up residence. Over the years, Reclamation has discussed various means of moving fish through the system (Liston *et al.* 1994, Fausch 2000).

A predator removal program in the secondary channel was studied and implemented in the early 1990s (Liston *et al.* 1994) and continued through the decade. Predators were washed into fyke nets, seined, and dip netted out during times when the secondary channel was drained. Striped bass were the main predatory species and fish up to 700 mm TL were removed. Other abundant predators at the facility include ictalurids, centrarchids and gobiids. Stomach analyses of some of these fish have yielded Chinook salmon (*Oncorhynchus tshawytscha*), delta smelt (*Hypomesus transpacificus*), threadfin shad (*Dorosoma petenense*), and other spp. (Liston *et al.* 1994). In recent years, predator removal activities have slowed because of logistics and the length of time the facility is down to complete the fish removal effort. In 2004, a new predator removal method using carbon dioxide (CO_2) was approved for study. This method would not reduce daily salvage and could prove to be more efficient, safer for employees and fish, and less labor

intensive than the current predator removal method. This project was divided into five phases and summaries of Phases 1–4 (completed in Sept. 2007) are included below.

Phase 1: Literature Review

During Phase 1 of our research we reviewed previous studies that focused on anaesthetization and the physiological impacts of CO_2 on fish in order to determine initial guidelines to follow prior to our laboratory studies and help us understand what type of behavior to expect when fish are exposed to CO_2. In addition, the review gave us a better understanding of additional factors (pH, alkalinity, temperature, fish size, fish spp.) that needed to be taken into consideration for our lab studies.

Phase 2: Water Chemistry

In Phase 2 of our project we performed water quality analyses in a lab setting.

A. Relationship Between CO_2 Concentration, pH, and Alkalinity

The relationship between alkalinity, CO_2 concentration ($[CO_2]$), and pH was examined and demonstrated that $[CO_2]$ ranging from <10–1000 mg/L could be reached and that the relationship between $[CO_2]$ and pH shifts with differing alkalinity. This allowed us to develop $[CO_2]$ vs. pH curves for water of different alkalinities.

B. Rate of CO_2 Dissipation

Our examination of the rate of CO_2 dissipation, and subsequent rise in pH, demonstrated that CO_2 could be easily removed from the water with aeration or agitation and that the addition of CO_2 did not cause an irreversible chemical reaction.

C. Effect of Dry Ice Block Size on CO_2 Sublimation Rate

The effect that the size of dry ice block has on sublimation rate and uptake into the water was examined. The chemical composition of the dry ice was assumed to be consistent throughout the block. It was determined that, within the size range, we use about 4.2–5.2% of each dry ice block every minute in water at 11.67 ºC and 4.9 m deep.

D. Behavior of Fish Exposed to CO_2

The final component of Phase 2 was to determine at what $[CO_2]$ striped bass, delta smelt, Chinook salmon and Sacramento splittail (*Pogonichthys macrolepidotus*) become disoriented (Initial Loss of Equilibrium, ILE) and begin floating "belly-up" (Total Loss of Equilibrium, TLE). These trials allowed us to determine that striped bass (341.3 ± 33.0 mm FL, n = 50) reached ILE in less than 10 min when the $[CO_2]$ in the water was 50 mg/L or greater and reached TLE in less than 10 min with a $[CO_2]$ of 200 mg/L and greater.

One hundred percent survival for 96 h was observed for striped bass that were exposed to $[CO_2]$ of <10, 50, 100, 150, and 200 mg/L for 20 min. Striped bass that were exposed to $[CO_2]$ of 250 mg/L and 300 mg/L for 20 min showed 80% survival for the 96 h observation period. The length of time needed for each spp. of fish to regain equilibrium at each $[CO_2]$ will be determined in future trials.

Phase 3: Flume Studies

Controls were completed in which there was no CO_2 injection into the water. We separately inserted various groups and sizes of striped bass, Chinook salmon, Sacramento splittail, and delta smelt into the TFCF oval flume. A 2-hp axial flow electric water pump was used to achieve appropriate water velocities (0.22 ± 0.04 m/s, n = 20) in the flume and a chiller was used to maintain proper water temperature (± 0.5 ^{o}C). An aluminum perforated plate (6.35-mm holes) was installed in the flume (15° to waterflow) at the end of one of the straight sections, in order to mimic the secondary bypass. A dip net was attached downstream to catch fish that had succumbed to the CO_2 and passed through the bypass. A small wooden board (0.61 m by 0.61 m) was also placed at the head of the straight section of the flume to provide a shady retreat for the fish and prevent them from willingly swimming with the current past the screened bypass.

These control trials were completed in order to demonstrate that, without anaesthetization, the fish being tested would not swim with the current and past the screened bypass during the experimental time limit (15 min). After the control trials had been completed we ran trials in which CO_2 was injected into the flume from a pressurized cylinder until the desired [CO_2] (100–200 mg/L) was reached. Fish were then inserted and time was recorded from the initial injection until each fish moved with the current past the bypass.

Phase 4: Pilot Test

Phase 4 consisted of a pilot study to demonstrate that predators could be moved through the bypasses using CO_2 and pulsed flows. Rather than drawing down the secondary channel to capture fish, we experimented with recovering fish in simultaneous HT/sieve net (SN) collections following a 30 min CO_2 treatment.

A control sample (no CO_2 injected) was obtained in the same manner as the treatment sample 4 h earlier in the day. The fish obtained in both samples were identified, measured, and counted in order to compare spp., sizes and numbers between groups.

This pilot study provided an initial assessment of CO_2 and supports that this may be an effective method for predator removals in the future; the CO_2 treatment removed more fish than the control treatment. In Phase 5 of this project we intend to apply the knowledge gained through our initial studies (Phases 1–4) in order to implement the combined use of CO_2 and pulsed flows as a predator removal technique at the TFCF.

Problem Statement

Predation may be significant within the primary bypass tubes and secondary channel because striped bass continue to reside within them. Removing these fish with the current methods is dangerous for employees, decreases daily salvage, and causes damage to the fish and/or fish mortality. An alternate method to remove predators is needed for the facility.

Goals and Hypotheses

Goals:

1. Reduce the number and average size of striped bass in the secondary system by removing large resident fish.

2. Increase survival of fish collected during the predator removal process.

3. Decrease the amount of time necessary to perform the predator removal process and minimize, or eliminate, facility downtime during predator removals.

4. Develop a predator removal technique that is safer for employees.

Hypotheses:

1. Collection efficiency and survival will be equal for [CO_2] of 0, 75, 150, 200, and 300 mg/L, over a 10-min exposure time.

2. The bypass tubes and secondary channel hold equal numbers of wild striped bass.

3. The proportion of injected fish removed by using the old and new predator removal method will be equal.

4. The proportion of fish that die or show signs of damage (*i.e.*, fungus, hemorrhaging) after 96 h will be equal for the old and new predator removal methods.

5. The amount of time to complete the old and new predator removal methods will be equal.

Materials and Methods

Phase 5: Implementation of CO_2 for Predator Removal

In the final phase of this project (Phase 5) we intend to apply the knowledge gained through our initial studies (Phases 1–4) in order to implement the combined use of CO_2 and pulsed flows as a predator removal technique at the TFCF. Phase 5 consists of components, and information that is learned will direct the next step in the research process. Phase 5 consists of two primary components: hydraulics and fisheries.

The examination of CO_2 injection on TFCF hydraulics will be investigated to determine a suitable location for CO_2 injection, develop a device for dry ice injection, determine whether dry ice is causing flow rate changes, determine how to stabilize flows and [CO_2] when using dry ice, determine if another method of CO_2 injection would provide more stable [CO_2] and flows, and produce a calculation that predicts peak [CO_2] from the amount of dry ice injected and the flow through each bypass tube in the secondary channel.

In order to investigate the response of TFCF fisheries to the combined use of CO_2 and pulsed flows, we must first determine where the majority of predators are congregating and the dose at which predators are most efficiently removed from the bypass tubes and secondary channel. Once predator location and an optimal dose are established, we will compare the alternative predator removal efficiency and survival to that of the current predator removal method.

A. Hydraulics

1) CO_2 Injection Method

In order to determine if an injection device, such as a slide or chute, should be used we will need to compare injection time and safety hazards for the use of the device to that for the injection where no device is used. The dry ice will be separated into four equal portions and kept in a cooler near the bypass tube into which it will be injected. The same amount of dry ice will be injected into each bypass tube regardless of whether or not a device is used. The time it takes two workers to introduce dry ice into all of the four bypass tubes, with and without an injection device, will be determined and compared. A safety evaluation will also be completed to identify all hazards that are encountered when injecting with and without a device. The safety evaluation for dry ice injections, with and without a device, will then be compared with the safety evaluation for the existing predator removal technique.

2) Hydraulic Changes When CO_2 is Injected into the Bypasses

In order to verify that CO_2 injection is causing flow rate changes and to develop a method to stabilize flows we must obtain flow measurements through each bypass tube after varying amounts of dry ice have been injected.

The control trial will be completed first by examining flow through each bypass tube without any dry ice injection. All velocity control (VC) pumps will be turned off and the secondary channel will be allowed to flow at about 0.57 cubic meters per second (cms) for 20 min. Flow measurements will be recorded for each of the four bypass tubes every 2 min. This same procedure will be completed after injecting 11.3, 22.7, 34, 45.4, 56.7 and 68 kg of dry ice into each bypass tube. After each treatment the secondary channel will be flushed for 5 min to remove the remaining dry ice inside the bypasses. A flow vs. time graph will be plotted for each bypass and amount of dry ice tested in an effort to illustrate the flow rate changes caused by CO_2 injection.

3) Stabilizing CO_2 Levels During 10-Minute Fish Dose

To determine if [CO_2] can be stabilized within the secondary system we are proposing to inject small amounts of dry ice throughout the 20-min dose time. In order to do this we must first drain the secondary channel so 1/5-hp pumps can be installed at the mouth of each bypass tube in order to obtain water for pH and CO_2 measurements. The secondary channel will then be backfilled and all VC pumps will be shut off in order to achieve a flow of 0.57 cms for 20 min. The same known amount of dry ice, for each bypass tube, will be broken into small pieces and injected into each bypass opening throughout the 20-min dose period. Carbon dioxide and pH measurements will be performed for each bypass every 5 min. This procedure will be repeated, with the same amount of dry ice per bypass tube, except that the ice will not be broken and will be injected, all at once, into each of the bypass tubes. A [CO_2] vs. time graph will be made for each treatment and bypass tube in order to determine if [CO_2] can be stabilized by injecting small amounts of dry ice throughout the dose time rather than all at once.

4) Alternate Forms of CO_2

It is possible that more stable [CO_2] and waterflows could be achieved by using gaseous CO_2 instead of dry ice. To investigate this, we would follow the same

procedures as described above, except that a pressurized CO_2 cylinder will be used to continuously inject CO_2 gas, with a stable flow (LPM), into the mouth of each bypass tube throughout the 20-min dose period. Flow through each bypass tube will be recorded every 2 min while [CO_2] and pH measurements would be taken every 5 min. These data will be used to construct a [CO_2] vs. time graph and a flow vs. time graph for the use of gaseous CO_2. These graphs will be compared to those developed for the dry ice injections in order to determine if using gaseous CO_2 allows for more stable [CO_2] and flows than using dry ice as a CO_2 source.

5) Predicting CO_2 Dose Fish are Exposed

A calculation will be produced to predict the peak [CO_2] in the bypass tubes depending on the amount of dry ice injected and the flow through each bypass tube. It will also be necessary for us to make a calculation that determines the amount of dry ice to add to the water in order to get to a target [CO_2] with a known bypass tube flow. When constructing these calculations we will need to take into consideration the percent of each dry ice block that is gassed off each minute and the efficiency of the gas dissolving into the water as a function of water temperature.

B. Fisheries

1) Predator Location

In order to determine the best location and method for CO_2 injection we must first figure out where striped bass are holding up within the secondary system. This will be done by injecting high doses of CO_2 (>200 mg/L) into two different areas of the secondary system (head of the secondary channel and entrance of bypass tubes) and comparing the number of predators removed. The secondary channel will be drained in order to install 1/5-hp submersible pumps at the mouth of each bypass tube which will be used to obtain water samples for CO_2 and pH measurements. The secondary channel will then be backfilled and all VC pumps will be shut off to achieve a flow of about 0.57 cms. The SN downstream of the secondary channel will be lowered before dry ice injection and will be used to evaluate the proportion and spp. of fish that are not successfully louvered into the HT (lost).

Dry ice (contained in a mesh bag) will first be lowered into the head of the secondary channel using a rope-pulley system to deliver the CO_2 to the mouth of the bypass tubes. Once a high [CO_2] is reached the flow in the secondary channel will be maintained at 0.57 cms for 20 min and then an empty HT will be opened and VC pumps will be turned on, for 10 min, in order to achieve a flow of 0.46–0.61 m/s and flush predators from the secondary channel into the HT. This HT sample will contain all of the predators that resided in the secondary channel but will not include any that were holding up in the bypass tubes.

After collection of the first sample, all VC pumps will be shut off again and a flow of 0.57 cms will be achieved. Dry ice will then be injected into the opening of each bypass tube until a high [CO_2] is reached in the secondary channel. A secondary channel flow of 0.57 cms will be maintained for 20 min after which an empty, HT will be opened and VC pumps will be turned on in order to flush the bypass tubes for 10 min at 0.46–0.61 m/s. This HT sample should contain all predators that resided within the bypass

tubes with the assumption that fish holding up in the secondary channel were previously removed.

If most of the predators are present in the secondary channel (1st HT sample), after the bypass tube mouths, then CO_2 injection in this area should be sufficient. If the majority of predators hold up in the bypass tubes (2nd HT sample) then CO_2 injection should take place at the mouth of these tubes in order to effectively remove these predators. If both locations hold predators then the CO_2 injection should take place at the bypass tube opening in order to collect predators from both locations.

2) Determining Optimal [CO_2] for a 10-Min Exposure

To determine the [CO_2] that is optimal for the removal of TFCF predators it is necessary to inject unique groups of ten striped bass for each of five consecutive predator removals exposing fish to five different [CO_2] (0, 75, 150, 200, and 300 mg/L). The order of the concentration tested will be randomized each day.

Groups consisting of 10 striped bass each will be given a distinct color/fin tag using a phototonic marking gun and BMX1000 phototonic marking formulation (NEWWEST Technologies, Santa Rosa, California). The secondary channel will be drained in order to install a 1/5-hp pump at the mouth of each bypass tube which will be used to obtain water samples for CO_2 and pH measurements. The secondary channel will then be backfilled, one group of 10 striped bass will be released, dry ice will be injected to obtain the target [CO_2] and a secondary and holding tank flow of 0.57 cms and 0.23 cms, respectively, will be achieved for 10 min. The SN downstream of the secondary channel will be lowered before fish injection and will be used to evaluate the proportion and spp. of fish that are not successfully louvered into the HT (lost).

After 10 min, VC pumps will be turned on in order to achieve a secondary flow of 0.46–0.61 m/s and flush the bypass tubes. Flushing time will be limited to 5 min as all CO_2 will have cleared the secondary channel by this time. The fish collected in the HT will be placed in a 3.6 m x 0.74 m x 0.76 m trough, equipped with O_2 and flow through Delta water, while the fish collected in the SN will be put into a 132.5-L garbage can containing Delta water. All fish will be identified and measured and the proportion of tagged fish recovered in each sample will be determined.

These methods will be repeated for [CO_2] of 0, 75, 150, 200, and 300 mg/L. Ninety-six h survival will be recorded for all recovered tagged striped bass. In order to detect the true probability of capture within 25%, it will be necessary to complete 30 replicates for each treatment (3 releases of 10 striped bass). The [CO_2] that is found to remove the greatest proportion of tagged striped bass (>90%), while maintaining acceptable survival (>90%) and least loss of fish (<10%), will be considered the optimal dose and will be used to compare the current predator removal technique to the proposed alternative predator removal method.

3) Current vs. Alternative Predator Removal Method

To evaluate the current and alternative predator removal techniques we will compare removal efficiency, survival, salvage loss time, cost, and safety. This will be done by performing five repetitions of each predator removal in which groups of 30 comparable sized striped bass (300–800 mm FL) will be given a distinct color/fin tag and released into the secondary system prior to each trial.

In order to perform the current predator removal technique we would first inject a distinctly marked group of 30 striped bass into the secondary channel. The secondary channel will then be drained, by closing all bypass tubes, in order to remove any readily available tagged predators using a dip or seine net. The SN downstream of the secondary channel will be lowered in order to collect any fish that are lost (not successfully louvered) during this predator removal process. The order that each bypass tube (1–4) will be flushed will be randomly determined and each tube will be individually opened for about 30 s while two biologists, equipped with waders and safety harnesses, hold a 6.35-mm mesh fyke net at the bypass mouth in order to collect flushed fish. After each of the bypass tubes has been flushed all bypasses will be opened and the secondary channel will be filled. The sieve net will be raised and any fish will be removed, identified, and measured. The proportion of tagged striped bass successfully recovered will then be determined. All tagged striped bass will be held for 96 h to determine survival. The time it takes to perform each trial will be determined in order to evaluate salvage loss due to secondary downtime. Time will be started the moment that HT flow is stopped until HT flow is resumed. The cost to perform each trial will also be estimated and will include labor, waders, harnesses, and price of the fyke, dip and seine nets. This process will be repeated until five repetitions of the current predator removal method are completed. Five replicates were chosen due to the fact that we are only interested in seeing differences greater than 25% between capture efficiencies of the two methods.

The evaluation of the alternative predator removal technique involves using the [CO_2] that was previously determined to be optimal for the removal of striped bass from the secondary channel. The secondary channel will be drained in order to install 1/5-hp pumps at the mouth of each bypass tube to provide water samples for CO_2 and pH measurements. The secondary channel will then be backfilled and all VC pumps will be turned off in order to achieve a secondary flow of 0.57 cms. The SN will be lowered and a distinctly marked group consisting of 30 striped bass will be injected into the secondary channel. Dry ice will then be introduced (location to be determined) until the optimal [CO_2] is reached. A secondary flow of 0.57 cms will be maintained for 10 min. After this time period, an empty HT will be opened and VC pumps will be turned on in order to flush the bypass tubes at 0.46–0.61 m/s. The proportion of tagged striped bass successfully louvered into the HT while using the optimal [CO_2] will be determined. All successfully recovered tagged striped bass will be held to determine 96 h survival. The time it takes to perform the alternative predator removal method will be determined by starting the timer when flow into the HT ceases and stopping the timer when HT flow is resumed. This will allow us to determine salvage loss due to secondary downtime. A cost of performing the alternative method will be estimated and will include dry ice costs, titration cells, pH meter, pumps, hoses, extension cords, and labor. This procedure will be repeated until five repetitions of the new predator removal technique are completed. Five replicates were chosen due to the fact that we are only interested in seeing differences greater than 25% between capture efficiencies of the two methods.

After completing the necessary replicates for both the current and alternative predator removal methods we will be able to make the appropriate comparisons between predator removal efficiency, predator survival, salvage loss time, cost and safety. This will allow us to determine which method is most effective and should be implemented as a TFCF predator removal technique.

Data Analyses

Carbon dioxide concentration in the secondary channel vs. time will be graphed for each of the three dosing techniques (large blocks, small blocks, gas). This graph will provide information on how stable the concentration of CO_2 stays with time. Logistic regression will be used to see if a significant capture-dose response exists within the range of 0–300 mg/L and if this is influenced by water temperature. A probability-capture curve will be used to determine the probability of capture within 25% for each [CO_2] being tested (*i.e.*, 0, 75, 150, 200, and 300 mg/L) using Probit analysis with a logit link function. A probability-survival curve will be used to determine the probability of 96 h post survival within 25%. Contingency tables will be used to compare the proportion of injected fish removed using the old and new predator removal methods. Contingency tables will be used to compare the proportion of wild striped bass collected in the bypass tubes and secondary channel. Contingency tables will also be used to compare the proportion of fish that die or show signs of damage after 96 h for each treatment. The average time needed to complete the old and new predator removal methods will be compared using a t-test.

Coordination and Collaboration

This study will be coordinated with the TFCF staff, Tracy Technical Advisory Team (TTAT), and California Department of Fish and Game (CDFG). Participation and inclusion of research-related updates will be provided at regularly scheduled TTAT and Central Valley Fish Facilities Review Team (CVFFRT) meetings.

Endangered Species Issues, "Take" Considerations

Winter-run Chinook salmon, steelhead trout (*O. mykiss*), and delta smelt may be encountered during these experiments. If this occurs, these fish will be immediately documented, returned to the Delta, and reported to all appropriate agencies.

Dissemination of Results (Deliverables and Outcomes)

A draft report for peer review and for TTAT covering Phases 1–4 will be completed in January 2010 and March 2010, respectively. The final phase (Phase 5) will be worked on during the next 2 years and a draft report for peer review and for TTAT will be completed by December 2010 and March 2011, respectively. The primary deliverable will be an article published in the Tracy Volume Series. Updates will also be provided at TTAT and CVFFRT meetings. Additionally, information will be gained on the successes and limitations of alternate predator removal techniques at the TFCF. This knowledge will help guide future development and implementation of predator removal procedures at the TFCF and other fish facilities.

Literature Cited

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